Impaired enteroendocrine development in intestinal-specific Islet1 mouse mutants causes impaired glucose homeostasis

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1The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; 2Division of Gastroenterology and Nutrition, Department of Pediatrics, Philadelphia, Pennsylvania; 3Department of Pathology and Laboratory Medicine, Philadelphia, Pennsylvania; and 4Perelman School of Medicine at the University of Pennsylvania, Department of Genetics and Institute for Diabetes, Obesity, and Metabolism, Philadelphia, Pennsylvania

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Terry NA, Walp ER, Lee RA, Kaestner KH, May CL. Impaired enteroendocrine development in intestinal-specific Islet1 mouse mutants causes impaired glucose homeostasis. Am J Physiol Gastrointest Liver Physiol 307: G979–G991, 2014. First published September 11, 2014; doi:10.1152/ajpgi.00390.2013.—Enteroendocrine cells secrete over a dozen different hormones responsible for coordinating digestion, absorption, metabolism, and gut motility. Loss of enteroendocrine cells is a known cause of severe congenital diarrhea. Furthermore, enteroendocrine cells regulate glucose metabolism, with the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) playing critical roles in stimulating insulin release by pancreatic β-cells. Islet1 (Isl1) is a LIM-homeodomain transcription factor expressed specifically in an array of intestinal enteroendocrine cells, including incretin-expressing cells. To examine the impact of intestinal Isl1 on glycemic control, we set out to explore the role of intestinal Isl1 in hormone cell specification and organissiology. Mice with intestinal epithelial-specific ablation of Isl1 were obtained by crossing Villin-Cre transgenic animals with mice harboring a Isl1loxP allele (Isl1mT model). Gene ablation of Isl1 in the intestine results in loss of GLP-1, GIP, cholecystokinin (CCK), and somatostatin-expressing cells and an increase in 5-HT (serotonin)-producing cells, while the chromogranin A population was unchanged. This dramatic change in hormonal milieu results in animals with lipid malabsorption and females smaller than their littermate controls. Interestingly, when challenged with oral, not intraportional glucose, the Isl1 intestinal-deficient animals (Isl1mT) display impaired glucose tolerance, indicating loss of the incretin effect. Thus the Isl1mT model confirms that intestinal biology is essential for organism physiology in glycemic control and susceptibility to diabetes.

The term “incretin hormone” refers to hormones secreted from the small intestine upon glucose challenge that are responsible for potentiating the effect of insulin in systemic glucose control (25, 51). The two known incretins glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are secreted from L and K cells (2), respectively, although they can also be found in overlapping cell populations (29). Antibody neutralization or loss of the corresponding hormone receptors leads to hyperglycemia (33). Exogenous administration of GLP-1 analogs or inhibitors of DPP IV, the enzyme responsible for rapid GLP-1 breakdown in the bloodstream, is utilized in the treatment of type 2 diabetes (36). Furthermore, increased GLP-1 levels have been implicated in the rapid glycemic control that is observed after gastric bypass surgery (16). The importance of incretin function has been further highlighted by genome-wide association studies of patients with type 2 diabetes, which have identified single nucleotide polymorphisms in the receptor for GIP (64). While the important role of the intestine in metabolic disease is now recognized (6), it is still unknown if alterations in intestinal gene function itself contribute to the pathogenesis of obesity or diabetes.

Several transcription factors have been implicated in the development of the enteroendocrine cells. Neurog3, as revealed by lineage tracing, is expressed in the precursor cell to all enteroendocrine cells (39). Furthermore, loss of Neurog3 in mice results in a loss of most enteroendocrine lineages (39, 44, 52), similar to the reports of congenital diarrhea in humans (71). Subsets of enteroendocrine cells are specified by NeuroD5 (56, 57), Insm1 (27), FoxA1/2 (43, 75), Pax4, and Pax6 (4, 43). Some transcription factors act as activators and repressors of endocrine cell fate such as PTEN (63), Nkx2.2 (15), and Arx (4, 19).

Interestingly, human mutations in ARX have been also been associated with cases of severe, even fatal, congenital diarrhea (41). In the mouse pancreas, Arx is required for the specification of α-cell fate in the islets of Langerhans (10, 11, 31) and acts downstream of Isl1 (46). Isl1 (Is1l) is a member of the LIM homeodomain transcription factor family (37), promoting expression of both somatostatin and preproglucagon in endocrine cell lines (45, 72) and whose expression is lost in FoxA1/2 intestinal mutant animals with impaired enteroendocrine development (75). Is1l has important functions in the heart, brain, and hindlimb (23, 42, 48) with Isl-expressing cells marking multipotent cardiac progenitors (7). Is1l is also required for the formation of both the exocrine and endocrine compartment of the pancreas (1, 18).

In the intestine, Is1l expression is restricted to enteroendocrine cells (13), although its function in the intestine has yet to

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Fig. 1. Islet1 (Isl1) is rarely expressed in chromogranin A (CGA) or serotonin (5-HT)-positive enteroendocrine cells. A–C: immunohistochemistry for Isl1 in duodenal crypts (arrows) and villi (asterisks) in postnatal day (P)7 (A) and 4-wk-old (B) and 4-mo-old (C) animals. A and C have an eosin counterstain. D: immunohistochemistry for Isl1 (brown, arrow) and CGA (blue, asterisk). E: percentage of Isl1-positive/CGA-positive cells in the duodenum. F: immunohistochemistry for Isl1 (arrows). G: immunofluorescence for 5-HT (Serotonin; red arrows). H: merge of Isl1 (brown) and 5-HT (red) cells. I: immunofluorescence with goat anti-somatostatin (SST; green). J: immunofluorescence with rabbit anti-CgA ab85554 (red). K: merge of SST (green) and CgA (red). Duodenal tissue from 4- to 5-wk-old animals in D and F–K.
be explored. Here, we find that Isl1 is expressed in a subset of enteroendocrine cells that are marked by GLP-1, GIP, somatostatin (SST), gastrin, and ghrelin. In intestinal-specific Isl1mut mouse mutants, these subpopulations are lost, leading to failure to thrive and lipid malabsorption in neonatal mice. Furthermore, without the presence of incretin hormones, Isl1mut mutant mice are not able to maintain normal glucose homeostasis during oral glucose tolerance tests (OGTT).

**MATERIALS AND METHODS**

**Animals.** The derivation of theloxP-flanked Isl1 conditional allele (Isl1loxP) and the Villin-Cre mouse lines has been reported previously (24, 69). These mice were maintained on a mixed background (CD1, 129, and C57BL/6) and were cared for and handled according to a protocol approved by the Children’s Hospital of Philadelphia’s Institutional Animal Care and Use Committee. Primers for genotyping alleles used in this study were 5′-GGTCTCTGGACATCCCAT-3′.

Fig. 2. Isl1 is not expressed in tuft, Paneth, or goblet cells. Immunofluorescence for DCAMKL1, marker of tuft cells (red in A and C), and lysozyme (Lys1; red in D and F). The white arrows in A and C designate a DCAMKL1-positive cell. Immunohistochemistry for Isl1 (brown) in B, C, E, and F and G–I. The brown arrows throughout designate nuclear, epithelial Isl1 staining. The white asterisk in B and C designates an Isl1-positive cell in the lamina propria. Goblet cells are marked by Alcian blue stain in G–I. I also has a hematoxylin and eosin stain. All tissue is duodenum from 4- to 5-wk-old animals except as designated in G–I.
Fig. 3. Isl1 is expressed in somatostatin, glucagon-like peptide-1 (GLP-1), ghrelin, glucose-dependent insulinotropic polypeptide (GIP), and CCK cells. Left: Isl1 expression (brown). Left middle: hormone expression with SST red in A, GLP1 green in B, ghrelin (GHR) green in C, GIP green in D, and CCK green in E. Right middle: Isl1 and hormone merge. Far right: graphs demonstrating the overlap of Isl1 and hormone-coexpressing cells related to the Isl1 population and the respective hormone population.
TGT-3' and 5'-TCAACAGTAAAATCTCTCCCTCCC-3' for Isl1loxP and 5'-GCGGCATGGTGCAAGTTGAAT-3' and 5'-CGTTCCAG-GCATCAACGTTT-3' for Cre. Daily weights were measured at mid-day after using toe marking to identify animals. Statistical significance for growth curves was determined using repeated-measures ANOVA in Graphpad PRISM software. Unless otherwise noted, animals were analyzed at 4–5 wk of age.

**Immunohistochemistry and immunofluorescence.** Tissues were fixed in 4% paraformaldehyde overnight at 4°C and embedded in paraffin or optimal cutting temperature freezing medium, and 8-μm
sections were collected. Slides were subjected to microwave antigen retrieval in 10 mmol/l sodium citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% H₂O₂ in PBS for 15 min, and sections were blocked with CAS Block reagent (Invitrogen). The sections were incubated with primary antibodies overnight at 4°C and appropriate secondary antibodies for 2 h at room temperature. Immunohistochemical detection was performed with the VECTASTAIN ABC kit (Vector Laboratories) and diaminobenzidine tetrahydrochloride as the substrate. For immunofluorescence, secondary antibodies were directly conjugated to Cy3 or Cy2 and incubated for 4 h at room temperature. The primary antibodies used were as follows: goat anti-somatostatin (1:3,000; Santa Cruz sc7819), rabbit anti-somatostatin (1:50; Invitrogen 18-0078), anti-grelin (1:200; Santa Cruz sc10368), anti-Isl1 (1:50; Developmental Studies Hybridoma Bank 39.4D5 and 40.2D6), rabbit anti-5-hydroxytryptophan (5-HT/serotonin; 1:50,000; ImmunoStar 20080), rabbit anti-chromogranin A (anti-CGA; 1:3,000; DiaSorin 20085), rabbit anti-CGA (1:200; Abcam ab85554; or 1:500; Abcam ab15160), mouse anti-GLP-1 (1:500; Abcam ab26278), anti-cholecystokinin (anti-CCK; 1:100; Santa Cruz sc21617), anti-GIP (1:250; Santa Cruz sc23554), anti-DCAMKL1 (1:100; Abcam ab37994), and anti-lysozyme (1:3,000; Dako A0099). Sections were stained with hematoxylin and eosin, Oil Red O, and alkaline phosphatase according to standard protocols. Oil Red O staining was performed using frozen sections. Hormone-positive cells from different regions of the intestine were counted and normalized to the respective epithelial area of the same or adjacent sections yielding cell numbers per millimeter squared tissue area. Epithelial area was measured with Aperio Image Analysis System. At least three control and three mutant animals (4–5 wk of age) were utilized for each hormone analysis in the intestine.

**Real-time RT-PCR analysis.** All tissue dissections were performed in cold 1× PBS, and tail snips were taken for genotyping. Total RNA was extracted in TRizol (Invitrogen). Oligo-dT, Superscript Plus, and other required reagents were used to synthesize cDNA. PCR reactions

![Image](http://ajpgi.physiology.org/)

**Fig. 5.** Isl1int females have reduced growth but normal histology. Representative images of immunohistochemistry for Isl1 expression in control (A, top, arrows) and Isl1int mice (A, bottom) demonstrating loss of epithelial expression although mesenchymal expression remains. Hematoxylin and eosin (B and C) and alkaline phosphatase (D) stains in 4-wk-old control (top) and Isl1int mice (bottom). Ileum is shown in C. All other images are duodenum. Growth curves for male (E) and female (F) control (black solid line) and Isl1int (red dashed line) mice.
were set up using Sigma-Aldrich SYBR Green JumpStart Taq in a Stratagene Mx3005P Real-Time PCR machine. At least four biologic replicates were performed for each genotype. Expression was normalized to the housekeeping gene Tbp (TATA-box binding protein), with control and Isl1int expression compared as fold change. Primer sequences are available upon request.

Glucose tolerance tests and glucose-stimulated insulin secretion. Following a 16 h fast, 3-mo-old animals (n = 7 per group) received oral gavage or intraperitoneal injection of 2 mg/g body wt glucose in sterile PBS. Blood glucose was measured using an automatic glucometer (One Touch Ultra; LifeScan, Milpitas, CA) at 0, 15, 30, 60, 90, and 120 min after gavage/injection. For the glucose-stimulated insulin secretion (GSIS), serum was collected at 0, 2, 5, 15, and 30 min after gavage/injection and placed on dry ice. Insulin levels were determined in duplicate using the Crystal Chem Ultra Sensitive Mouse Insulin ELISA Kit. Statistical significance was determined using a Student’s t-test for each time point, and the area under the curve was calculated in GraphPad PRISM software with the mean time zero glucose-test for each time point, and the area under the curve was calculated.

Serum and stool lipid analysis. Mice were fasted for 16 h after 10–12 wk on a high-fat diet and then plasma or serum was collected for use with the Wako NEFA-HR(2) kit, Cayman Chemical Triglyceride Kits. The fecal fat analysis was performed at The Children’s Hospital of Philadelphia Metabolomic Core.

RESULTS

Isl1 is expressed in the endocrine cells of the small intestine, primarily in CGA-negative cells. We examined the expression of Isl1 in the duodenum and ileum by immunohistochemistry in adolescent mice after weaning (4–5 wk of age). In both the proximal and distal small intestine, a strong nuclear stain was detected in scattered epithelial cells in addition to mesenchymal tissue. Isl1-positive cells were present in both the crypts and villi of the epithelium in a pattern suggestive of enteroendocrine cells (Fig. 1, A–C) and similar to its expression in the gastric epithelium (13). Isl1 was not expressed in goblet cells (Alcian blue−), Paneth cells (Lysozyme+), or tuft cells (DCAMKL1+; Fig. 2, A–I).

CGA and 5-HT (serotonin) are found in a high percentage of enteroendocrine cells (62, 68). Although initially proposed as a pan-endocrine marker, CGA is not expressed in all enteroendocrine cells (9, 61, 63, 73). Interestingly, Isl1 expression overlapped with <3% of CGA-positive cells (Fig. 1, D and E) and only one 5-HT+/Isl1+ cell was found among over 1,000 5-HT cells counted in the duodenum (Fig. 1, F–H).

To determine which enteroendocrine cells express Isl1, we performed dual label immunofluorescence studies using antibodies to multiple gut hormones. SST has been demonstrated to define an independent population of enteroendocrine cells that do not coexpress CCK, GIP, or GLP-1 (21). Furthermore, CGA is only expressed at very low levels in GIP and GLP-1

Fig. 6. Isl1int nursing pups have fat malabsorption. Oil Red O staining for neutral fat in stool samples of P7 (A) and P28 (B) animals from control (left) and Isl1int (right) littermates. Oil Red O staining of tissue samples in P7 ileum (C) and P7 colon (D) from control (left) and Isl1int (right) littermates.
expressing cells of the upper small intestine (29). To confirm previous reports that SST and CgA rarely overlap (61), we tested two different SST antibodies and three different CgA antibodies (Fig. 2, I–K). The two anti-SST antibodies exhibited near complete overlap, indicating that the two reagents indeed recognize the Somatostatin protein. Next, we performed double-immunostaining with goat anti-SST and the different rabbit anti-CgA antibodies. We counted over 150 highly expressing SST-positive cells in the duodenum and found only 2 cells with faint CgA-positive staining. In the CgA-positive population, we found rare CgA-positive cells that demonstrated low levels of SST-positive immunoreactivity.

By counting all Isl1-positive cells and hormone positive cells, we determined that Isl1 is expressed in 62% (±7.3%) of SST-positive cells, 84% (±3.9%) of GLP-1-positive cells, 68% (±10.3%) of ghrelin-positive cells, and 32% (±8.7%) of GIP-positive cells (Fig. 3, A–D). Isl1 also overlaps with the CCK population, although technical difficulties did not allow us to determine the precise percentage of coexpression (Fig. 3E).

**Hormone cell populations are altered with loss of epithelial Isl1.** The colocalization of nuclear Isl1 protein in a specific subset of enteroendocrine cells raised the possibility that this transcription factor might be required for the differentiation or maintenance of enteroendocrine cells. We crossed Villin-Cre with Isl1loxP/loxP animals to ablate Isl1 in the intestinal epithelium. For simplicity, the experimental Isl1loxP/loxP;Villin-Cre mice will be referred to as Isl1int in this article. These mice did not demonstrate any increased mortality or reduced fertility. As expected, epithelial expression of Isl1 was lost in Isl1int mice while mesenchymal expression of Isl1 was maintained, as determined by immunohistochemistry (see Fig. 5A). By quantitative RT-PCR (qRT-PCR), Isl1 mRNA expression was reduced in the mutant animals, although not significantly when analyzing the whole intestine, likely due to the remaining mesenchymal Isl1 mRNA expression (data not shown).

To determine if Isl1 is required for the differentiation or maintenance of enteroendocrine cells, we examined the cell populations in which Isl1 is predominantly expressed in the duodenum in 4- to 5-wk-old animals. First, we evaluated the
expression of various hormones by qRT-PCR (Fig. 4). Transcripts for somatostatin, preproglucagon, and GIP were reduced at least 20-fold in Isl1int mice. Expression of CCK was significantly reduced as well, by about fivefold. In addition, ghrelin mRNA expression also trended down by twofold, but the difference was not statistically significant (data not shown). Expression of CGA, which is rarely coexpressed with Isl1 (see above), was not changed (data not shown). Messenger RNA levels of tryptophan hydroxylase 1 (Tph1; encodes serotonin/5-HT), in which cells Isl1 is also not expressed, were mildly, but significantly, elevated by 1.6-fold.

Next, we determined expression of the relevant hormones by immunohistochemistry and calculated the number of positive cells per epithelial area (Fig. 4). Similar to the qRT-PCR results, the number of SST-, GLP-1 (protein product of the preproglucagon mRNA in intestinal L-cells)-, and GIP (K cells)-positive cells was dramatically reduced, with very few hormone-positive cells detectable in Isl1int mice. The CCK population was also significantly downregulated, while the number of ghrelin-positive cells was reduced but not significantly. The number of 5-HT (serotonin) cells again was significantly upregulated by 1.7-fold, corresponding to the change in Tph1 mRNA levels. The CGA population was unchanged (data not shown).

Loss of epithelial Isl1 results in neonatal fat malabsorption and poor weight gain. To determine if growth was affected by the loss of intestinal epithelial Isl1, mice were weighed daily until weaning at postnatal day (P)21. Beginning in the second week of life, female Isl1int mutant mice were significantly smaller than their littermate controls (Fig. 5, E and F; males $P$ value $/H_{11005} = 0.381$; females $P$ = 0.05). Using hematoxylin and eosin staining to compare the morphology of the tissue, we found no gross difference between control and mutant animals at 4 wk of age (Fig. 5, B and C). Also, the brush border was

![Graphs showing GSIS and AUC for OGTT and IPGTT](http://ajpgi.physiology.org/)
intact in Isl1int animals as determined by alkaline phosphatase stain (Fig. 5D).

Both neonatal male and female Isl1int mutant mice had soft stools. As fat malabsorption has been noted in both Neurog3 and Arx intestinal gene deletion models (19, 52), we tested stool and tissue for excess neutral lipid by Oil Red O staining. At P7, when neonatal pups are nesting on high-fat milk, there was an obvious increase in Oil Red O staining in the stool of the female and male Isl1int mutant mice, suggesting a decreased ability to absorb lipid (Fig. 6A). Furthermore, Oil Red O accumulated in epithelial cells of the ileum and in the colonic epithelium cells closest to the lumen in Isl1int mutant mice (Fig. 6, C and D). This phenotype disappeared when animals were switched to the standard rodent chow diet after weaning, a diet that has a very low-fat content. There was no detectable difference in the Oil Red O staining of the stool (Fig. 6B) or epithelial tissue of control and Isl-1int mutant mice at P28, 1 wk after weaning (data not shown).

Adult Isl1int mice process lipid normally on a high-fat diet. Since neonatal Isl1int mice had lipid malabsorption on a high-fat nursing diet, we tested if the lipid malabsorption recurred in adults fed a high-fat diet. We placed both male control and Isl1int 3-mo-old mice on a high-fat diet (45% calories from fat) and tested stool smears for Oil Red O weekly. Even after 12 wk on a high-fat diet, no Oil Red O was present in the stool of Isl1int or control adult mice (data not shown). Weight gain was carefully followed over this time period, but there was no significant difference in body weight overall (Fig. 7A).

Since mice lacking CCK have an impaired metabolic profile (109), we tested both fat/lean content of the stool and tissue for excess neutral lipid by Oil Red O staining. As fat malabsorption has been noted in both Neurog3 and Arx intestinal gene deletion models, we tested if the lipid malabsorption recurred in adults fed a high-fat diet. We placed both male control and Isl1int 3-mo-old mice on a high-fat diet (45% calories from fat) and tested stool smears for Oil Red O weekly. Even after 12 wk on a high-fat diet, no Oil Red O was present in the stool of Isl1int or control adult mice (data not shown). Weight gain was carefully followed over this time period, but there was no significant difference in body weight overall (Fig. 7A).

Since mice lacking CCK have an impaired metabolic profile on a high-fat diet (47), we tested both fat/lean content of the mice by NMR analysis as well as food intake, stool output, heat production, activity, and respiratory exchange ratio during a 24-h monitoring period after 14–16 wk on the high-fat diet. The control and Isl1int mice demonstrated no difference in fat/lean composition (Fig. 7B), food or water intake (Fig. 7C), total stool output (Fig. 7D), or total lipid content of the stool (Fig. 7E). The Isl1int mice also did not have any impairment in activity, heat production, or respiratory exchange rate (data not shown). The intestine was stained with Isl1 and SST, which confirmed continued ablation of Isl1 and reduction in SST-expressing cells (data not shown).

**Intestinal Isl1 mutants display impaired oral glucose tolerance.** With the dramatic loss of the incretin hormones GLP-1 and GIP, we hypothesized that oral glucose tolerance would be impaired in Isl1int mice. Therefore, we tested the response of the Isl1int mice compared with littermate controls with an OGTT in 3-mo-old animals. Isl1int female mice were significantly more hyperglycemic 15 min after administration of the glucose bolus while Isl1int male mice were significantly more hyperglycemic at 30 and 60 min after an oral glucose bolus (Fig. 8, A and B). This hyperglycemia returned to normal levels as the oral glucose load was cleared. The area under the curve was significantly larger in Isl1int males compared with control littermates (P value = 0.045; Fig. 8E), although we did not observe a difference in this measure in females. To determine if the glucose intolerance was due to a loss of the incretin effect, mice were also challenged with an intraperitoneal glucose tolerance test (IPGTT). The control and Isl1int mice did not demonstrate any difference in glucose levels during IPGTT, as expected (Fig. 8, C–E).

Since the loss of the incretin hormones should affect insulin levels, we performed GSIS during both OGTT and IPGTT in male mice (seven 3- to 4-mo-old male mice of each genotype were available for testing). During OGTT, significantly less insulin was secreted in the Isl1int mice overall as demonstrated by area under the curve (Fig. 8, G and I; P = 0.007). No difference in insulin levels was detected during IPGTT (Fig. 8, H and I).

**DISCUSSION**

Our study describes a novel mouse model of congenital diarrhea and impaired glucose homeostasis. Without Isl1 in the small intestine epithelium, neonatal mice have poor growth and lipid malabsorption. Furthermore, the loss of incretin signaling from the intestine leads to glucose intolerance and reduced insulin secretion. Several subsets of enteroendocrine cells are lost. GLP-1, GIP, and somatostatin-expressing cells are nearly completely eliminated, while CCK-positive cells are significantly reduced and 5-HT (serotonin) cells are significantly upregulated. Interestingly, there is no overall change in the CGA population, which is expected since Isl1 is minimally expressed in this cell population.

Ultimately, with the loss of these enteroendocrine populations, the Isl1int mice have impaired oral glucose tolerance. The pancreatic insulin response is more robust after oral glucose compared with intravenous glucose administration (25, 51). Mice with gene ablation of both the GIP receptor and the GLP1 receptor have helped to elucidate the role of both of these incretin hormones in glucose homeostasis, with impaired glucose tolerance in GLP1 receptor null (GLP1R−/−; Ref. 65), GIP receptor null (GIPR−/−; Ref. 54), and the double incretin null mouse (DIRKO; Ref. 32). In the intestine, however, loss of GIP itself does not impair glucose tolerance (58). It is difficult to ablate only the intestinal GLP-1 protein, as it is derived from the preproglucagon transcript produced in both the intestine and pancreas where glucagon is produced from the same gene.
within the intestine, and its loss could cause increased motility (53). Somatostatin is a general inhibitor of hormone secretion and in the tissue, particularly the ileum. Both GLP-1 and lipid malabsorption with both excess fat present in the stool that the other endocrine hormones affected in the small intestine is not the only source of GLP-1 (66) or Isl1int glucose tolerance curves of that the other endocrine hormones affected in Isl1int mice also contribute to glucose homeostasis. The signaling of the incretin hormones and other enteroendocrine hormones is quite complex; additionally, the central nervous system is also a source of GLP-1 (17).

Glucose homeostasis has also been tested in intestinal-specific Neurog3 mutant mice (52), demonstrating that loss of all the enteroendocrine cell population leads to poor weight gain and relative hypoglycemia after oral glucose challenge. However, since these mice have lean body mass, the hypoglycemia is difficult to interpret from the perspective of specific hormonal secretion. Neurog3 mutations are known to play a role in congenital diarrhea in humans (71) and are linked to malabsorptive diarrhea with neonatal diabetes (60). Like Neurog3, Isl1 is involved in early pancreatic development (1, 28). A pancreatic-specific deletion of Isl1 in mice leads to loss of pancreatic endocrine mass, including islet β-cells, and subsequent hyperglycemia (18). Islet1 has been implicated in the pathogenesis of type 2 diabetes through genome-wide association studies (3, 22, 67, 74, 76), which would be consistent with its role in both the pancreas and intestine.

In addition to neurog3 mutations, mutations in proprotein convertase 1/3 (PC1/3) have been described as a cause of malabsorptive diarrhea and obesity in humans (49). PC1/3 processes many different hormones in the brain, pancreas, and intestine, where it is responsible for cleaving proglucagon into its multiple protein products including GLP-1 and GLP-2 (70). A mouse model of PC1/3 deficiency results in growth failure and endocrinopathies (77). Although it is suspected that multiple enteroendocrine hormones are altered with PC1/3 deficiency, the exact defect is unknown (38). Thus the lack of the proglucagon products in the intestine may be involved in both the altered glucose homeostasis and the etiology of the diarrhea seen in Isl1int mouse mutants and human patients with PC1/3 mutations.

The diarrhea present in the neonatal Isl1int mice is related to lipid malabsorption with both excess fat present in the stool and in the tissue, particularly the ileum. Both GLP-1 and GLP-2 are involved in lipid absorption, with opposing effects (53). Somatostatin is a general inhibitor of hormone secretion within the intestine, and its loss could cause increased motility (12). Furthermore, CCK regulates the secretion of pancreatic enzymes and is involved with lipid absorption (47). Thus the combined loss of these hormones likely leads to lipid malabsorption on a high-fat neonatal diet. The lipid malabsorption, however, did not recur when Isl1int mice were placed on a high-fat diet. This pattern is reminiscent of patients with PC1/3 mutations who generally require intravenous nutrition in the first year of life but who can maintain their growth with full enteral feeds around toddler age (49). Since adult Isl1int mice did not have any lipid malabsorption or metabolic derangement on a high-fat diet, either Isl1 is required for different hormonal populations in neonatal mice, different hormones contribute to lipid absorption in the neonatal and adult periods, or a specific lipid is present in the milk for neonatal mice that is not present in a Chow high-fat diet.

In summary, Isl1 is required for the specification of a subset of enteroendocrine cells leading to lipid malabsorption in neonatal mice and the loss of the incretin response in adults. This model is unique in its description of an intestinal-specific defect impairing glucose tolerance. Furthermore, it elucidates the role of Isl1 in enteroendocrine development (Fig. 9) and diarrheal disease. Mouse models such as Isl1int are important for studying the link between nutrient absorption and metabolic disease.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES
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INTESTINAL ISL1 REGULATION OF GLUCOSE HOMEOSTASIS


